although the accuracy would probably suffer. In the case of conjugated oils a widely accepted method is still not available although the hydrogenation procedure is valuable as a research tool and the modified Rosenmund-Kuhnhenn Method described by Planck and associates at the Southern Regional Laboratory seems to offer definite possibilities. As for determination of composition the old, much used thiocyanogen, hexabromide, and tetrabromide procedures are giving ground to the more modern spectral methods.

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Letter to the Editor

Recently Cama, Chakrabarty, Hilditch, and Meara (1, 2) have published evidence in opposition to Kartha's criticism of the crystallization procedures for estimation of glyceride types in natural fats (3, 4, 5). The writer has made a careful study of the article by Cama *et al.* and has communicated with Dr. Kartha concerning it. There appear to be some items which are open to question.

1. Cama and colleagues record the results of the analysis, by the "crystallization" method, of several fat mixtures of supposedly known composition. The authors say that the accordance between observed and calculated values is generally good, but they express uncertainty over the composition of some of the ingredients because of the possibility that oxidative changes have occurred. In this writer's opinion there is reason, in addition, to question the calculated compositions of all the fat mixtures; consequently the accuracy with which the glyceride types were determined is in doubt. This uncertainty arises from the fact that the very same "crystallization" method used in the analysis of the mixture was employed in the analysis of the various ingredients thereof. Any errors inherent in this crystallization technique would appear in both the analysis of the ingredients and that of the mixture, and the latter analysis could therefore be erroneous even though the observed and calculated values were in perfect agreement. If the proportions of the various glyceride types were not accurately determined when the ingredients were analyzed, the same or similar errors could appear when the mixtures were analyzed because the analytical procedure was the same. Therefore even good correlation cannot in this case be taken as evidence that the "crystallization" method is accurate.

2. Cama *et al.* have collected data from the literature showing the proportions of GS_3 in samples of several natural fats, determined by both the "oxidation" and "crystallization" procedures. On the basis of these data and on the assumption that the oxidation method gives correct values, they have concluded that GS_3 can accurately be determined by the crystallization method.

Kartha (3, 4) has cast some doubt on the reliability of the particular oxidation method employed for determination of GS_3 in the work cited by Cama. If the procedure is unreliable, it cannot be employed as the whole method or any part of a method used as a criterion of the accuracy of the crystallization method.

Of the 14 fats cited by Cama *et al.* nine were analyzed by a "crystallization" procedure which includes an oxidation step. Errors inherent in the "oxidation" procedure will therefore appear in the "crystallization" procedure, and the two cannot with confidence be placed in contrast.

Of the remainder of the fats three, namely, stillingia tallow, cocoa butter, and palm oil (Belgian Congo), were each analyzed by a "crystallization" procedure including no oxidation step. Two, coconut oil and palm kernel oil, were probably analyzed in a similar manner, but this is uncertain because the reference is to unpublished data.

In every case, save one in which the proportions of GS_3 were the same by either method, analysis by the "oxidation" procedure resulted in a higher value for the content of GS_3 than that obtained by the crystallization method. When the values are corrected to compensate for the differences in the S content of the whole fat, the divergence is even greater.

This relationship is in complete accord with Kartha's statement (4) that determination of GS_3 by the oxidation method used in these instances may result in error and that the error will be positive. The evidence advanced by Cama *et al.* therefore tends to prove that the "oxidation" procedure is subject to error and does not prove that either of the "crystallization" procedures is accurate. The writer readily concedes that low temperature fractionation followed

by ester fractionation of the concentrates may result in accurate values for the GS_3 content. It also appears likely that Kartha's revised oxidation procedure may be used satisfactorily in analysis of the fractions.

3. Cama and colleagues have compiled data showing that for a variety of seed fats the proportions of simple triglycerides found by crystallization procedures form "a remarkably regular sequence" parallel to the proportions in the whole fat of their constituent acids arranged in descending order. The simple triglycerides are of several kinds, both saturated and unsaturated. The authors indicate that the regularity of the sequence, together with the presumption that the GS_3 was accurately determined by the crystallization procedure, constitutes strong evidence that GU_3 as well as GS_3 can be accurately determined by the same method.

The fact that the sequence is regular (with a few exceptions) cannot, in this writer's opinion, be construed to mean that all the data are accurate. There is sufficient margin between individual analyses in many instances to permit gross error to exist without the fact being apparent.

4. Cama *et al.* have resolved a mixture of GS_2U, GSU_2, and GU_3 into what appear to be the concentrates from which it was prepared. These consisted of "OS_2" composed of GS_2U and GSU_2 and "OL_2" composed of GSU_2 and GU_3.

Once again, as in section one above, the composition of the mixture cannot be accepted as accurate because the ingredients were analyzed by the same method as that under examination. One would expect little difficulty in separating a mixture, into the various simpler mixtures of which it is comprised, by the same procedure used to prepare the ingredient mixtures.

It should be pointed out that although Boekenoogen *et al.* (6) recommended the use of acetic acid in the oxidation procedure in 1950, it was first disclosed by Kartha in his doctoral thesis written in 1949. The summary of the thesis was not published until 1951, which probably accounts for the misapprehension (2).

The writer is in agreement with Dr. Hilditch that Kartha's procedure should be thoroughly tested. Until this is done, neither the analytical procedure nor the theory of glyceride structure dependent upon it can be evaluated.

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December 9, 1954

Letter to the Editor

I appreciate very much the courtesy of R. J. Vander Wal, who has been so kind as to let me see his letter to you (1) prior to its publication. I regret that I do not find myself in agreement with the arguments put forward by him to show that the paper by Cama *et al.* (2) is based upon unsound premises.

1. Dr. Vander Wal questions the calculated compositions of the fat mixtures used because the "very same crystallization method" was used both in the analysis of the mixtures and of the constituents thereof. This is surely an overstatement: the components of the mixtures were relatively simple compared with the complex mixtures of these which were employed. The application of the same general technique to glyceride mixtures of such widely varying build is, in my opinion, no valid objection to the argument. Rather, if the crystallization procedure were as inaccurate as Dr. Kartha has asserted, it would require an extreme series of coincidences to result in any accordant results being obtained in the experiments described by Cama *et al.*

2. Dr. Vander Wal further rules out any of the data in which trisaturated glycerides were at any stage of the "crystallization" procedure determined by our procedure of oxidation, which Dr. Kartha (3) alleged to be inaccurate. I have so far seen no reason to accept Dr. Kartha's criticisms and am satisfied that any advantage in his preferred oxidation procedure is confined to relatively slight, if any, alterations in the determined proportions of trisaturated glycerides.

3. Dr. Vander Wal "readily concedes that low temperature fractionation followed by ester-fractionation of the concentrates may result in accurate values for the GS_3 content." In this he appears to differ from Dr. Kartha, who has condemned the crystallization procedure and who (4) announced his failure to separate by crystallization a very simple mixture of oleodistearin and triunsaturated glycerides.

As mentioned in my previous letter to your Journal (5), a very large number of natural fats have now been examined by the "crystallization" procedure, and it is now possible to plot graphs of the contents of glycerides containing one, two, or three groups of a variety of acids (saturated, oleic, linoleic, linolenic, elaeostearic, ricinoleic, and several others). Whatever acid may be considered, the experimentally found points of glycerides containing one, two, or three of its groups are distributed about curves which are of precisely the same shape for each individual acid and can indeed be superimposed. The content of glycerides containing one group of a particular acid reaches a maximum (85-90%) when that acid forms exactly one-third of the total acids, and the content of glycerides containing two groups of the acid reaches a similar maximum when the acid forms exactly two-thirds of the total acids. Since my previous letter was written, typical curves of this kind have been published (6); a completely detailed account of glyceride structure as revealed by the "crystallization" procedure used by my associates and by other workers (which is too lengthy to be dealt with in a communication to a scientific journal) will appear in due course in a book of mine now in the printer's hands.

I regret that Dr. Vander Wal has not considered in his letter two criticisms of Dr. Kartha's work which I made (5) and which appear to me to demand serious attention:

1. According to Kartha's data obtained by his "revised oxidation procedure" for 23 natural fats (rang-